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MOLECULAR IDENTIFICATION OF FEATHERS FROM A COMANCHE ARTIFACT

Kevin C. R. Kerr and William Voelker

*This study investigates the source species for feathers incorporated into two Comanche artifacts known as **hi kodako**. We attempted to determine the originating species using a molecular approach—specifically DNA barcodes—because the feathers were modified in appearance. We tried three different DNA extraction methods on feathers from one complete **hi kodako** and a remnant feather from a second artifact, each more than 100 years old. The silica membrane extraction method was most successful in this study. While tradition holds that only corvid feathers should be used, our results suggest that **hi kodako** are actually composed of feathers from multiple species, which in this case included ferruginous hawk (*Buteo regalis*), domestic chicken (*Gallus gallus*), and northern raven (*Corvus corax*). This information bears important cultural significance and provides a greater degree of accuracy to the historic ethno-ornithological record.*

Key words: Native Americans, Comanche, corvids, feathers, ancient DNA, DNA barcodes

*Se estudiaron las especies a las que pertenecen las plumas empleadas en dos artefactos Comanche conocidos como **hi kodako**. Debido a la modificación del aspecto de las plumas, el método de determinación de las especies se basó en el uso de códigos de barras de ADN. Se probaron tres métodos diferentes para la extracción de ADN a partir de las plumas de un **hi kodako** entero y de una pluma que quedaba en un segundo artefacto, ambos de más de 100 años de antigüedad. La extracción en membrana de sílice fue la más exitosa. Pese a que la tradición sostiene que sólo se usaban plumas de córvidos, según nuestros resultados los **hi kodako** contenían plumas de varias especies, incluidas las de *Buteo regalis*, *Gallus gallus*, y *Corvus corax*. Esta información tiene un importante significado cultural y provee un mayor grado de exactitud a los registros históricos etno-ornitológicos.*

Introduction

The Comanche, known to themselves as *Numunuh*—"The People," originate from the Shoshonean group of the Uto-Aztecan branch of the Aztec-Tanoan linguistic stock of indigenous people of North America. Separating from the Eastern Shoshoni who inhabited the upper reaches of the Platte River in eastern Wyoming, the group destined to become known as Comanche migrated southward onto the Southern Plains during the late A.D. 1600s. Once inhabiting the plains, the Comanche mastered the horse, brought back to the Americas by the Spanish, and became the most influential of the Great Plains horse and buffalo cultures. Comanche warrior accoutrements pertinent to the horse culture were of great spiritual and ceremonial importance. The avian and mammalian components of these items are species specific due to the cultural perception of special attributes or "medicine" the species possess (Boster and D'Andrade 1989; Diamond 1966; Forth 2004; Nabhan 2000).

The *titsiwai* or Comanche lance is historically one of the most iconic and spiritually significant physical manifestations of the warrior ethic, as has been maintained through oral history. Certain features of the *titsiwai* make the Comanche lance unique among the Great Plains horse and buffalo cultures. The *hi kodako* (literal translation: corvid necklace) is one such *titsiwai* attachment unique to the Comanche. According to tradition, the *hi kodako* is composed of no less than 24 crow, raven and/or magpie feathers individually woven onto a sinew thread. The feathers are then cut to a uniform length and attached to the shaft of the lance by wrapping the “necklace” around and over itself. Depending on the prowess of the warrior, one to as many as four *hi kodako* could be attached to the lance. The traditional belief is that the spirit of the corvid, through the energy of the feathers, could greatly assist the warrior in battle. Thus, only corvid feathers were originally used in the construction of the *hi kodako*. However, some feathers recovered from *hi kodako* artifacts have been thought to actually originate from turkey vulture (*Cathartes aura*), a species considered taboo in traditional Comanche culture and unlikely to be incorporated into such attachments. Because of these implications, the accurate identification of feathers from historic *hi kodako* bears cultural significance.

Whole feathers, such as from headdresses, may be identified by comparison to those from study skins. But *hi kodako* are composed of feathers that have been cut up and otherwise altered, which impedes morphological identification. Alternatively, feathers can be a viable source of DNA, whether naturally shed (Hogan et al. 2007; Rudnick et al. 2007), collected fresh from live birds (Kerr et al. 2007), or sampled from museum study specimens (Ellegren 1994), allowing for genetic-based identification. Although DNA has been extracted from feathers from museum specimens 100 years old or more (Leeton et al. 1993), acquiring DNA samples from *hi kodako* feathers presents a further challenge because they have been cut, modified, and fragmented, and most are lacking the calamus, which is the base of the feather shaft and known to be a reliable location for genetic material (Horváth et al. 2005).

We attempted to recover DNA from pith extracted from the feather shafts of a *hi kodako*, known as the Parker/Burnett artifact, to facilitate identification. A feather separated from a second and better preserved *hi kodako*, the Saupitty artifact, was also sampled subsequently. From the extracted DNA, we sequenced the mitochondrial gene cytochrome *c* oxidase subunit 1 (COI). The 5' region of COI has recently been employed as a “DNA barcode,” a short, standardized gene segment used as a marker for species identification (Hebert et al. 2003). There is a relatively complete library of DNA barcodes for North American birds that previous studies have successfully used to identify fragmentary bird remains (Dove et al. 2008; Kerr et al. 2007), which demonstrates that COI is an ideal gene to target for species identification. We tried three different extraction methods, though results were limited. Two of the 26 feathers sampled from the Parker/Burnett artifact could be identified, but DNA from the Saupitty feather was more readily obtained.

Methods

The Parker/Burnett artifact was composed of 62 feathers strung together with sinew and thread (Figure 1). Both the proximal and distal ends of the

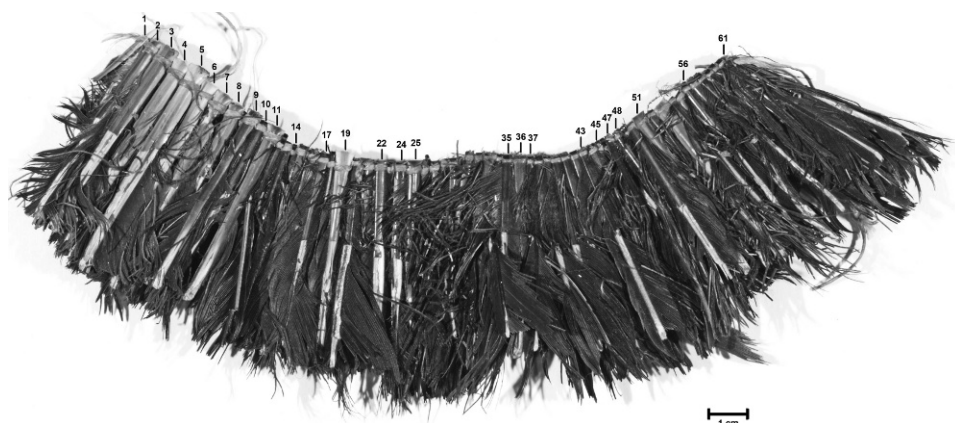


Figure 1. Dorsal view of the Parker/Burnett artifact. Feathers are numbered consecutively from left to right but only those sampled in this study are labeled. Photograph by Rick Turner.

feathers were discarded when the *hi kodako* was made. The artifact was estimated to be more than 120 years old and had been displayed over a fireplace in a private ranch house for more than 60 years. The Saupitty artifact was estimated to be at least 110 years old and was better curated. It was of comparable size to the Parker/Burnett artifact but permanently affixed to a nine-foot lance, hence only one feather that had previously detached during handling was available to sample (Figure 2). The authenticity of both artifacts had been carefully documented through the collection of historic images archived at Sia, The Comanche Nation Ethno-Ornithological Initiative (Cyril, Oklahoma).

To sample the Parker/Burnett artifact, pith was scratched away from the fragmented distal ends of the feather shafts using a flame-sterilized scalpel blade and fine tipped forceps. Due to the cultural importance of this item, great care was taken to limit damage to the specimen. The three DNA extraction methods included spin columns with silica membranes ($n=10$), magnetic beads ($n=10$), and a Chelex-based method ($n=14$). To maximize coverage, different feathers were sampled for each method, with some overlap for control purposes (Table 1). For silica membrane extractions, we used the NucleoSpin tissue kit (Macherey-Nagel, Düren, Germany); for magnetic beads, we used the ChargeSwitch

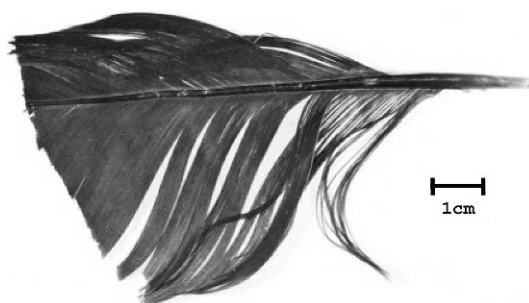


Figure 2. Dorsal view of the feather separated from the Saupitty artifact.

Forensic DNA Purification kit (Invitrogen, Carlsbad, California); and for the Chelex-based method we used the DryRelease method described by Hajibabaei et al. (2005). Both commercial kits were used according to the manufacturer's instructions. Additionally, two feathers were sampled consumptively: the Saupitty feather and a single feather removed from the Parker/Burnett artifact (feather #50 in Figure 1). The barbs were removed from the feather shafts, which were cut into 5 mm sections. Four segments from each feather were used for extraction using the NucleoSpin kit, and four using the DryRelease method.

Extractions were performed in a dedicated research and development laboratory in the Biodiversity Institute of Ontario at the University of Guelph. No avian samples had been previously processed in that lab, reducing the risk of contamination. All kits were new and unused. Pipettes were cleaned prior to use and only new, barrier tips were used to handle liquid reagents. For a positive control, we used fresh contour feathers from a *Passer domesticus* specimen so that cross-contamination could be easily identified.

New PCR primers were designed to amplify a short fragment from the barcoding region of COI, ranging from 84 to 90 bp in length, depending on the primer combination. Two degenerate forward primers were designed: Com1F (5'-TAATYGGAGGATTGGAACTG-3') and Com2F (5'-CATAATYGGAGGATTGGAAAC-3'). The two reverse primers corresponded directly to a COI sequence from either *Corvus brachyrhynchus*, ComCr_R1 (5'-CTCCTTCTAGCCTC TTCCACAG-3'), or *Cathartes aura*, ComVu_R1 (5'-CCTACTCCTACTAGCCTC TTCTACAG-3'). Although these primers were designed specifically to target the aforementioned species, their application turned out to be more universal. The contents of the PCR mix followed that outlined by deWaard et al. (2008). Two microliters of DNA template were added to each sample. PCR samples were run using the following thermal cycle program: One cycle at 94°C for 1 min, five cycles of 94°C for 1 min, 45°C for 40 s, and 72°C for 1 min, thirty-five cycles of 94°C for 1 min, 51°C for 40 s, and 72°C for 1 min, and lastly one cycle of 72°C for 5 min. PCR products were sequenced bi-directionally on an ABI 3730xl DNA Analyzer. Contigs were assembled from forward and reverse reads when possible using SEQUENCHER, version 4.5 (Gene Codes). Recovered sequences were identified using BLASTN (Altschul et al. 1990) available from the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

Sequencing results for the Parker/Burnett artifact were limited. Only three sequences of avian origin were recovered, including two from the same feather. The sequences corresponded to feathers numbered 3 (n=2) and 24 (n=1) in Figure 1. All three sequences were derived from Nucleospin extractions and were obtained using the primer combinations Com1F/ComCr_R1 or Com1F/ComVu_R1. We had no success using either the magnetic bead or Chelex extraction methods. The two sequences from feather #3 were assembled into an 88 bp contig with 5 ambiguous base calls. The lone sequence for feather #24 was 57 bp with no ambiguous calls; however, some base call reads were ambiguous at both the 3' and 5' ends of the sequence due to sequencing impurities, which is

<i>Buteo regalis</i>	ACTGTGTCCTCATAATCGGCGCCCCGACATAGCCTTCCCAGCATAAACACATAAGCTTCTGACTACTTCTCCATCCTTTCTC
<i>B. jamaicensis</i>	C.....T.....C...
<i>B. lagopus</i>	T.....C...
<i>B. platypterus</i>	C.....T.....C.....C...
<i>B. swainsoni</i>	C.....C.....C...
Feather #3	NNN.....NN.....
<i>Cathartes aura</i>	.T.A..C.....T.....T.....A..T.....A.....C.....C.....C..A
<i>Corvus corax</i>	..A....T..A....T..T....A..T....A....A.....C..A..C..A..C..T
<i>C. brachyrhynchus</i>	..A..C..T..A..G..T..T....A.....A.....C.....C..A..C.....C..T

<i>Gallus gallus</i>	ACTAGTCCCGCTTATAATCGGTGCCCGCCAGACATAGCATTTCCCGGCATAAATAACATAAGCTTCTGACTCTCTCCCTCCCTCCTTCTCTT
Feather #24	
<i>Cathartes aura</i>	.T.....A.....C.....C.....T.....T..A..A.....C.....A..T..C..A.....A
<i>Corvus corax</i>	T..T..A..G..T.....T.....A.....C.....C.....A.....A.....A.....
<i>C. brachyrhynchus</i>	T..A..G..T.....A..A.....C.....A.....

Figure 3. Alignments of COI sequences for query sequences from feather #3 (top) and feather #24 (bottom). Each header row features the most similar species to the query identified using BLASTN. Other species represented include four hawks (*Buteo* spp.), turkey vulture (*Cathartes aura*), and two corvids (*Corvus* spp.). Nucleotides identical to those in the header row are indicated with a dot. The two query sequences are highlighted in bold text. GenBank accession numbers for the reference sequences are: DQ433390 (*Buteo regalis*), DQ434504 (*B. jamaicensis*), AY666502 (*B. lagopus*), DQ432787 (*B. platypterus*), DQ433391 (*B. swainsoni*), AY666510 (*Cathartes aura*), DQ432868 (*Corvus corax*), and DQ432866 (*C. brachyrhynchus*).

why the sequence is short. These sequences were identified as belonging to ferruginous hawk (*Buteo regalis*) and domestic chicken (*Gallus gallus*), respectively (Figure 3). The feather from the Saupitty artifact yielded DNA more readily. All eight extraction attempts based on PCR amplification were successful, and the resultant sequencing attempts yielded undifferentiated COI sequences up to 90 bp in length matching northern raven (*Corvus corax*). In comparison, all extractions from feather #50 were unsuccessful.

No cross-contamination of the positive control was detected in the sequencing results. However, in a small percentage of samples, either human or bacteria sequences were identified. The origin of the human contaminants could not be determined.

Discussion

Our findings suggest that the *hi kodako* artifacts investigated here were composed of feathers from multiple species. The sequencing results from the Saupitty feather confirm that corvid feathers were indeed used, even if only in part, while the results from the Parker/Burnett artifact implies that dark feathers from other species were incorporated into these ornaments out of necessity. Unfortunately, we cannot confirm or rule out the use of vulture feathers since most of the feathers from the examined artifacts yielded insufficient genetic material and remain unidentified.

Although the sequences we produced were relatively short, the reliability of minimalist barcodes to identify older specimens with degraded DNA has been previously demonstrated (Hajibabaei et al. 2006). In birds, intraspecific COI sequence variation is generally reported to be very low and thus should not impede barcode-based identifications (Kerr et al. 2007). Of greater concern is limited variation between sister species. Very young pairs of species are often separable by only a few nucleotides (Kerr et al. 2007), or none at all in those that hybridize (Aliabadian et al. 2009), which can pose a serious challenge for

identification when using only a short stretch of DNA. Luckily, this does not appear to be a valid problem for the North American members of the query taxa. However, the sequence from feather #3 later proved to be a match for two Old World species (i.e., common buzzard, *Buteo buteo*, and upland buzzard, *Buteo hemilasius*) that were not available at the time of the original analysis (Yoo et al. 2006), but since these species do not occur in North America, they do not affect our interpretation of the data.

Most of the potential sources for laboratory error that could compromise the results of this study may be ruled out. For example, the amplification of pseudogenes is of limited concern. Though stop codons may not necessarily occur across such a small fragment of DNA, which impairs our ability to detect pseudogenes, there is a low chance of recovering nuclear copies of mitochondrial DNA in ancient specimens (Haddrath and Baker 2001). Since avian specimens had not been handled in the laboratory prior to this analysis, the chances of these sequences being the result of contamination is highly unlikely, though independent replication would have strengthened these results. Although, DNA from domesticated species, including *Gallus gallus*, has been documented in PCR reagents (Leonard et al. 2007), which could compromise the validity of that species identification.

Parts of the feather thought to be most DNA-rich (i.e., the calamus) were missing from these artifacts (Horváth et al. 2005). However, a recent study revealed that genetic material can be recovered from throughout the feather shaft (Rawlence et al. 2009), suggesting that the limited feather remains should not necessarily limit DNA availability. But the difference in recovery rates between the Parker/Burnett artifact and the Saupitty feather is notable. Recovery of ancient DNA is always difficult and obviously is dependent on having a specimen that contains suitable material (Cooper 1994). Handt et al. (1994) argue that “[m]ost archeological and paleontological remains do not contain endogenous DNA that can be amplified via PCR but do contain DNA from exogenous sources such as bacteria and fungi, as well as contaminating DNA from contemporary humans.” While the DNA yield significantly decreases as a specimen ages, even from tissue sources richer than feathers (Lee and Prys-Jones 2007), the Parker/Burnett and Saupitty artifacts were nearly the same age. However, even in recently collected feathers, it has been shown that PCR success declines dramatically with feather condition (Hogan et al. 2007). Thus, it would appear that the degraded condition of the Parker/Burnett artifact, due mostly to a long duration of improper storage (e.g., exposure to sunlight and humidity), was responsible for low success rates.

The results of this study offer insight into the composition of *hi kodako* artifacts and reveal that feathers from a variety of bird species other than corvids were indeed used, which in part explains past speculation over the origin of these feathers. The flexibility in Comanche culture could allow for substitute feathers to be used in the construction of the *hi kodako* as long as the substitutes were similar in appearance to the proper corvid feather and that at least some corvid feathers were utilized. However, the occurrence of vulture feathers in *hi kodako* remains unconfirmed. Further analysis of other preserved Comanche items might enhance the verbal history and cultural significance of these artifacts. This

study demonstrates the difficulty and limitations in obtaining DNA from poorly preserved artifacts, but also reinforces the value of comprehensive, standardized libraries of reference sequences for the identification of unknown materials and underscores the importance of DNA methods for anthropological studies.

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